*Original Article*

# Detection of Carbapenemase Genes by Molecular Method among Gram-Negative Bacilli Isolates from Tertiary Care Hospital in Dhaka, Bangladesh

**Mostofa HA[1], Shamsuzzaman SM[2], Hasan MM[3], Sultana S[4], Afroz S[5], Shormin M[6], Sultana SS[7]**

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**Abstract**

***Background****: Imipenem resistance in Gram-negative bacilli is a global epidemic that is increasing day by day. To warn this global epidemic, identification and ongoing surveillance of carbapenem-resistant genes among Gram-negative bacilli needed.*

***Objectives****: This cross-sectional study was performed to detect the imipenem resistant genes among Gram-negative bacilli isolated from different samples in Dhaka medical college hospital.*

***Methods****: About 300 samples (wound swab, urine, endotracheal aspirate, blood, and sputum) were collected from July 2015 to June 2016. Among them, 204 isolates were Gram negative bacilli. Eighty imipenem resistant Gram-negative bacilli were isolated by disc diffusion method. Among them, carbapenem resistant genes (blaNDM-1, blaKPC, VIM, IMP) were detected by PCR.*

***Result****s: A total 300 samples were analyzed. Out of 204 Gram negative bacilli,80 (39.21%) imipenem resistance was detected by the Disc Diffusion method. Out of 80 imipenem resistant organisms, 42 (52.5%) were positive for blaNDM-1, 6 (7.34%) were positive for blaKPC, 29 (36.25%) were positive for VIM, 13 (16.25%) were positive for IMP.*

***Conclusion****: This study illustrates the emergence of carbapenemase genes producing Gram negative bacilli isolates from patients. Close surveillance across all hospitals in Bangladesh is required.*

**Key Words:**
Carbapenemase, Drug Resistance,Genes, Gram-negative bacilli, Imipenem

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## Introduction:

Carbapenem drugs are the most valuable drugs for treating multi-drug resistant Gram-negative bacteria (MDR-GNB) infections. However, there has been a significant growth of carbapenem-resistant organisms that cause severe damage to public health.[1,2] Carbapenem resistance is mainly owing to the expression of a carbapenemase enzyme, efflux pump, or porin loss. Among these, the most important and difficult mechanism is the production of the carbapenemase enzyme, because it is present on mobile genetic elements, which are easily transferable from one bacterium to another bacterium such as *Pseudomonas spp.*, *Acinetobacter spp.*, *Escherichia coli* (*E. coli*), and *Klebsiella spp.*, which the World Health Organization (WHO) has designated as high priority organismsin 2017.[3,4] The major carbapenemase genes are bla-*Klebsiella pneumonia*carbapenemase (blaKPC), bla-oxacillin hydrolyzing enzymes-48 (blaOXA-48), bla-New Delhi metallo-â-lactamase (blaNDM), bla-Verona integron-mediated metallo-â-lactamase (blaVIM), and bla-active on imipenem (blaIMP), which are present globally and cause nosocomial infections. Many researchers have

1. Dr. Hasbi Ara Mostofa**,** Assistant professor, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka
2. Dr. SM Shamsuzzaman**,** Professor and Head, Department of Microbiology, Dhaka Medical College, Dhaka
3. Dr. Md Maniul Hasan**,** Assistant professor, Department of Surgery, Shaheed Suhrawardy Medical College, Dhaka
4. Dr. Sharmeen Sultana, Associate Professor, Department of Microbiology, Monno Medical College, Manikganj.
5. Dr. Samira Afroz, Assistant Professor, Ad-din Women's Medical College, Dhaka
6. Dr. Moonmoon Shormin, Assistant Professor, Shaheed Monsur Ali Medical College & Hospital, Dhaka
7. Dr. Sabia Shahin Sultana, Lecturer, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka

**Correspondence to:** Dr. Hasbi Ara Mostofa, Assistant professor, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka, Mobile: 01717921581, Email: hasbiaramostofa@gmail.com

studied various methods of carbapenem resistance detection including carbapenemases. [2,5,6]

In this current study, we want to detect carbapenem genes which are caused imipenem resistant among Gram negative bacilli in hospital in our country.

**Materials and methods:**

A total of 300 samples (wound swab, urine, endotracheal aspirate, blood, and sputum) were collected from July 2015 to June 2016 in Dhaka Medical College Hospital in a cross-sectional study. All the wound swab, urine, pus, and endotracheal aspirate samples were inoculated in blood agar and MacConkey agar media and incubated at $37^0$ Caerobically for 24 hours. Incubated plates were then examined for the presence of colonies of bacteria. 204-Gramnegative bacilli were isolated. Susceptibility to Imipenem of all isolates was done by Kirby Bauer modified disk diffusion technique using Muller Hinton agar plates, and zones of inhibition were interpreted according to CLSI guidelines (CLSI, 2015).Antibiotic disc imipenem (10ig) was used. The examinedclear zone of inhibition around the disc on the testorganisms were interpreted as resistant and sensitive. All strains were tested for antibiotic susceptibility by Disk Diffusion and were designated for Imipenem as susceptibleif the inhibition zone diameter was e"22 mm, intermediateif the inhibition zone diameter was 19–21 mm, and resistantif the inhibition zone diameter was d"18 mm, asrecommended by Clinical and Laboratory StandardInstitute (CLSI) (2015).[11] Carbapenemase genes such as *bla*NDM-1, *bla*KPC, *bla*IMP and *bla*VIM genes were detected by PCR using specific primers.

**Results:**

Of the total 300 samples, 204 (68%) gram negative bacteria were isolated. Out of 204 isolated Gram negative bacteria, 80 (39.21%) imipenem resistant strains were detected. Table I: Out of 80 imipenem resistant organisms, 42 (52.5%) were positive for *bla*NDM-1 detected by PCR. Of them, 10 (12.5%) were isolated from wound swab, 3 (3.75%) from urine, 25 (31.25%) from ETA, 2 (2.5%) from blood and 2 (2.5%) from sputum. Among 12 imipenem resistant *Esch.coli*, 6 (50%) were positive for *bla*NDM-1. Five (55.55%) of the 9 imipenem resistant *K.pneumoniae*, 3 (75%) of the 4 imipenem resistant *K.oxytoca*, 3 (50%) of the 6 imipenem resistant *Citrobacter freundii*, one (50%) of the 2 imipenem resistant *Enterobacter aerogenes*, 17 (86%) of the 20 imipenem resistant *Acinetobacter baumannii* and 7 (29.16%) of the 24 imipenem resistant *Pseudomonas aeruginosa* had NDM-1 encoding gene.

Table II: Out of 80 imipenem resistant Gram- negative bacteria, 6 (7.34%) were positive for *bla*KPC detected by PCR. Among 20 imipenem resistant *Acinetobacter baumannii*, 6 (30%) were positive for *bla*KPC. Among 36 imipenem resistant *Enterobacteriaceae*, 4 (11.11%) from wound, 2 (5.55%) from urine and 2 (5.55%) from ETA. Among 24 *Pseudomonas aeruginosa*, no KPC encoding gene was found.

**Table I**

Detection of NDM-1 gene by PCR among imipenem resistant Gram- negative organisms (N=80).

| Organism | Woundswab n (%) | Urine n (%) | ETA n (%) | Blood n (%) | Sputumn n (%) | Total n (%) |
|---|---|---|---|---|---|---|
| *Esch.coli*(N=12) | 2(16.67) | 1(8.33) | 3(25.00) | 0(0.00) | 0(0.00) | 6(50.00) |
| *K.pneumoniae*(N=9) | 2(22.22) | 0(0.00) | 1(11.11) | 1(11.11) | 1(11.11) | 5(55.55) |
| *K.oxytoca*(N=4) | 1(25.00) | 0(0.00) | 1(25.00) | 0(0.00) | 1(25.00) | 3(75.00) |
| *Citrobacter*(N=6) | 1(16.67) | 1(16.67) | 1(16.67) | 0(0.00) | 0(0.00) | 3(50.00) |
| *Enterobacter*(N=2) | 0(0.00) | 0(0.00) | 1(50.00) | 0(0.00) | 0(0.00) | 1(50.00) |
| *A .baumannii*(N=20) | 2(10.00) | 0(0.00) | 14(70.00) | 1(5.00) | 0(0.00) | 17(86.00) |
| *P. aeruginosa*(N=24) | 2(8.33) | 1(4.16) | 4(16.67) | 0(0.00) | 0(0.00) | 7(29.16) |
| Total | 10(12.50) | 3(3.75) | 25(31.25) | 2(2.50) | 2(2.50) | 42(52.50) |

N= Total number of bacteria,n= Total number of positive cases

**Table II**

*Detection of KPC gene by PCR among imipenem resistant Gram -negative bacteria (N= 80).*

| Organisms | Wound n (%) | Urine n (%) | ETA n (%) | Blood n (%) | Sputum n (%) | Total n (%) |
|---|---|---|---|---|---|---|
| *Enterobacteriaceae*(N=36) | 4 (11.11) | 2 (5.55) | 2 (5.55) | 0 (0.00) | 0(0.00) | 8 (22.22) |
| *Acinetobacter baumannii* (N=20) | 0 (0.00) | 0 (0.00) | 6 (30.00) | 0 (0.00) | 0(0.00) | 6 (30.00) |
| *Pseudomonas aeruginosa*(N=24) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0(0.00) | 0 (0.00) |
| Total | 4 (5.00) | 2 (2.5) | 8 (10.00) | 0 (0.00) | 0(0.00) | 14(17.50) |

N= Total number of bacteria,n= Total number of positive cases

Table III: Out of 80 imipenem resistant organisms, 29 (36.25%) were positive for VIM detected by PCR. Of them, 14 (17.5%) were isolated from wound swab, 13 (16.25%) from ETA and 2 (2.5%) from blood. Among 12 imipenem resistant *Esch.coli*, 2 (16.67%) were positive for VIM. One (11.11%) of the 9 imipenem resistant *K.pneumoniae*, one (33.33%) of the 3 imipenem resistant *Proteus vulgaris,* 3 (42.86%) of the 7 imipenem resistant *Citrobacter freundii*, 13 (65%) of the 20 imipenem resistant *Acinetobacter baumannii* and 12 (50%) of the 24 imipenem resistant *Pseudomonas aeruginosa* had KPC encoding gene.

Table IV: Out of 80 imipenem resistant organisms, 13 (16.25%) were positive for IMP detected by PCR. Of them, 4 (5%) were isolated from wound swab, 2 (2.5%) from urine and 7 (8.75%) from ETA. Among 12 imipenem resistant *Esch. coli*, 3 (25%) were positive for IMP. Eight (40%) of the 20-imipenem resistant *Acinetobacter baumannii* and 2 (8.33%) of the 24-imipenem resistant *Peudomonas aeruginosa* had IMP encoding gene.

**Table III**

*Detection of VIM gene by PCR among imipenem resistant Gram -negative organisms (N= 80).*

| Organisms | Wound n (%) | Urine n (%) | ETA n (%) | Blood n (%) | Total n (%) |
|---|---|---|---|---|---|
| *Esch.coli*(N=12) | 1 (11.11) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 2 (16.67) |
| *K.pneumoniae*(N=9) | 2 (25.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 1 (11.11) |
| *K.oxytoca*(N=4) | 1 (33.33) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| *Proteus spp*.(N=3) | 1 (33.33) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) |
| *A.baumannii*(N=20) | 3 (15.00) | 0 (0.00) | 8 (40.00) | 1 (10.00) | 13 (65.00) |
| *P.aeruginosa*(N=24) | 7 (29.16) | 0 (0.00) | 45(20.83) | 0 (0.00) | 11 (45.83) |
| Total | 14 (16.25) | 0 (0.00) | 13 (16.25) | 2 (2.50) | 27 (33.75) |

N= Total number of bacteria, n= Total number of positive cases

**Table IV**

*Detection of IMP gene by PCR among imipenem resistant Gram -negative organisms (N=80).*

| Organisms | Wound n (%) | Urine n (%) | ETA n (%) | Blood n (%) | Sputum n (%) | Total |
|---|---|---|---|---|---|---|
| *Esch.coli*(N=12) | 1 (8.33) | 2 (16.60) | 0 (0.00) | 0 (0.00) | 0(0.00) | 3 (25.00) |
| *K.pneumoniae*(N=9) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0(0.00) | 0 (0.00) |
| *K.oxytoca*(N=4) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0(0.00) | 0 (0.00) |
| *Citrobacter freundii*(N=6) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0(0.00) | 0 (0.00) |
| *Enterobacter aerogenes* (N=2) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0(0.00) | 0 (0.00) |
| *Acinetobacter baumannii*(N=20) | 2 (10.00) | 0 (0.00) | 6 (30.00) | 0 (0.00) | 0(0.00) | 8 (40.00) |
| *Pseudomonas aeruginosa* (N=24) | 1 (4.16) | 0 (0.00) | 1 (4.16) | 0 (0.00) | 0(0.00) | 2 (8.33) |
| Total | 4 (5.00) | 2(2.50) | 7 (8.75) | 0 (0.00) | 0(0.00) | 13 (16.25) |

N= Total number of bacteria, n= Total number of positive cases

## Discussion

Antimicrobial resistance has become a major health issue with many clinical isolates showing limited or no susceptibility to currently available antimicrobials (Lim *et al*., 2010). In this present study, out of 80 imipenem resistant Gram-negative bacilli, 52.5% were positive for NDM-1 gene. Of them, 12.5% were isolated from wound swab, 3.75% from urine, 31.25% from ETA, 2.5% from blood and 2.5% from sputum samples (Table 1). Previous study in DMCH by Khatun (2014), reported that 53.57% NDM-1 producers among the imipenem resistant Gram-negative bacilli. A previous study in Bangladesh, demonstrated that 3.5% NDM-1 producers among the imipenem resistant organisms (Islam *et al*., 2011). In another study, Farzana *et al*. (2013) reported that 22.8% NDM-1 positive among the imipenem resistant Gram-negative bacteria in Bangladesh. The findings of the present study revealed that the prevalence of *bla*NDM-1 gene in Gram-negative bacteria is increasing in Bangladesh. The increasing percentage of this new resistance mechanism might be due to healthcare associated acquisition of *bla*NDM-1 in hospitalized patients worldwide including Bangladesh (Struelens*et al*., 2010).

In the present study, out of 80 imipenem resistant Gram-negative bacteria, 17.5% were positive for *bla*KPC gene detected by PCR (Table 2). Previous study in DMCH by Khatun (2014) reported that 7.34% were positive for *bla*KPC detected by PCR, and all of them were only found in imipenem resistant *Acinetobactorbaumannii*. Another study in DMCH by Sattar (2016) reported that 21.62% were positive for *bla*KPC among imipenem resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca.* The highest prevalence of KPC producing organisms to date were identified mostly in the United States, Israel and in Greece (Poirel*et al*., 2010). In the present study, out of 36 imipenem resistant *Enterobacteriaceae*, 22.22% were positive for *bla*KPC gene. In contrast to the present findings, Shanmugam *et al.* (2013) in India reported that 67.4% isolates harbored *bla*KPC gene among imipenem resistant *Enterobacteriaceae.* This finding does not correlate to the present study because Shanmugam *et al.* (2013) observed KPC gene only in *Enterobacteriaceae*. The presence of this gene suggests the possibility of horizontal transmission, as this carbapenemase has been associated with mobile genetic element (transposons) which can be transferred from one bacterium to another (Gootz and Marra, 2008; Nass *et al*., 2008).

Current study observed that 36.25% VIM producers among imipenem resistant Gram-negative bacilli detected by PCR. A study by Begum (2015) revealed 15% VIM producers among imipenem resistant Gram-negative bacteria. Another study in DMCH by Sattar (2016) reported 16.22% VIM producers among imipenem resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca.* In the present study, 50% were VIM producers among imipenem resistant *Pseudomonas aeruginosa*. A study by Abedin (2016) in DMCH reported 46.87% VIM producers among imipenem resistant *Pseudomonas aeruginosa* which is close to the present findings. From Italy and Greece, 87.5% and 100% VIM-1 producers were identified respectively (Cornaglia*et al*., 2000; Tsakris*et al*., 2000). These findings were not in accordance with present findings because Cornaglia*et al*. (2000) and Tsakris*et al*. (2000) identified VIM-1 and all of them were only found in imipenem resistant *Pseudomonas aeruginosa* and most of the *Pseudomonas aeruginosa* isolated from ICU. But in the present study, VIM was detected in all the Gram-negative bacilli isolated from different wards of a tertiary care hospital. The reported prevalence of NDM-1 producing bacteria varies in different studies which might be due to geographical variations of such drug resistance pattern.

In the present study, out of 80 imipenem resistant Gram-negative bacilli, 16.25% were positive for IMP gene detected by PCR (Table4). A study by Begum (2015) in DMCH revealed 10% IMP producers among imipenem resistant Gram-negative bacteria which is close to the present findings. A recent study in DMCH by Sattar (2016) reported 13.51% IMP producers among imipenem resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca.* In the present study, 8.33% were IMP producers among imipenem resistant *Pseudomonas aeruginosa*. In contrast to the present findings, 96.15% IMP producing *Pseudomonas aeruginosa* were reported from China where all the sputum samples were collected from ICU patients (Chao *et al*., 2008).

## Conclusion:

Periodic review of the bacteriologicalprofile and antibiotic sensitivity pattern is highly essential.Antibiotic policy & infection control program should beincluded in every hospital to reduce this drug resistance. About 52.5% NDM-1 producer were detected among isolated carbapenemase producing Gram-negative bacilli. which are important in choosing empirical therapy, designing good antibiotic policies, updating local antibiotic guidelines for doctors, and in determining clinical treatment failure.

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